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Expression of Fas Ligand in Langerhans' Cell Histiocytosis: A Case Report of a Boy with Multisystem Involvement

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Previous reports of patients with Langerhans' cell histiocytosis have shown characteristics of osteolytic lesion, visceral involvement and organ dysfunction. We report a 2-year-old boy who was diagnosed as Langerhans' cell histiocytosis with a prominent hepatomegaly. X-Radiogram, computed tomography and magnetic resonance imaging revealed the osteolysis of the right iliac bone, the absence of the left eighth rib as well as the right mandible, and an enhancing mass in the left cerebellum. The data of radiography were highly suggestive of Langerhans' cell lineage. The presence of large CD1a-positive mononuclear cells associated with inflammatory cells in peripheral blood smear and bone marrow aspirate further confirmed the diagnosis. In addition, expressions of S100, CD25, CD68, CD80, CD86, and Fas ligand were identified on these cells by immunocytochemical staining. The results indicate that although these cells are activated Langerhans' cells, progression of the disease and the bone destruction could be mediated by the overt FasL expression of the cells. Am. J. Hematol. 61:256–261, 1999.

Key words: histiocytosis; Langerhans' cell; CT; MRI; Fas ligand; immunocytochemistry

INTRODUCTION

Langerhans' cells (LCs) are major histocompatibility complex (MHC) class II-bearing bone-marrow-derived cells mainly distributed in the epidermis of the skin [1]. These cells express CD1a on the cell surface and contain racket-shaped microstructure, Birbeck granules, in the cytoplasm [2–4]. They could engulf and process antigenic material. The antigen-bearing LCs will migrate from the epidermis to the paracortex of the draining lymph node and differentiate into $CD1a^-$/CD83+/CD86+ dendritic cells (DCs) or CD1a+/CD83+/CD86+ interdigitating reticulum cells (IDCs) [5,6]. During the differentiation, LCs could rapidly upregulate MHC class II expression and co-stimulatory factors to complete antigen presentation to T cells, whilst losing their ability to process antigens and no longer containing Birbeck granule [5,6]. The in vitro culture of CD34+ progenitor cells and LCs confirmed that CD34+ progenitor cells could differentiate into LCs in the presence of tumor necrosis factor α (TNFα), granulocyte-macrophage colony-stimulating-factor (GM-CSF), and interleukin 4 (IL-4), and that LCs could differentiate into DCs or IDCs [7–9].

Langerhans' cell histiocytosis (LCH) is a disease characterized by the abnormal proliferation of histiocytes [10,11]. The disease can affect skin, bone, lymph node, spleen, liver, and central nervous system [12–15]. According to severity and visceral involvement of the disease, LCH has been named histiocytosis X, eosinophilic granuloma, Letterer–Siwe disease, Hand–Schüller-

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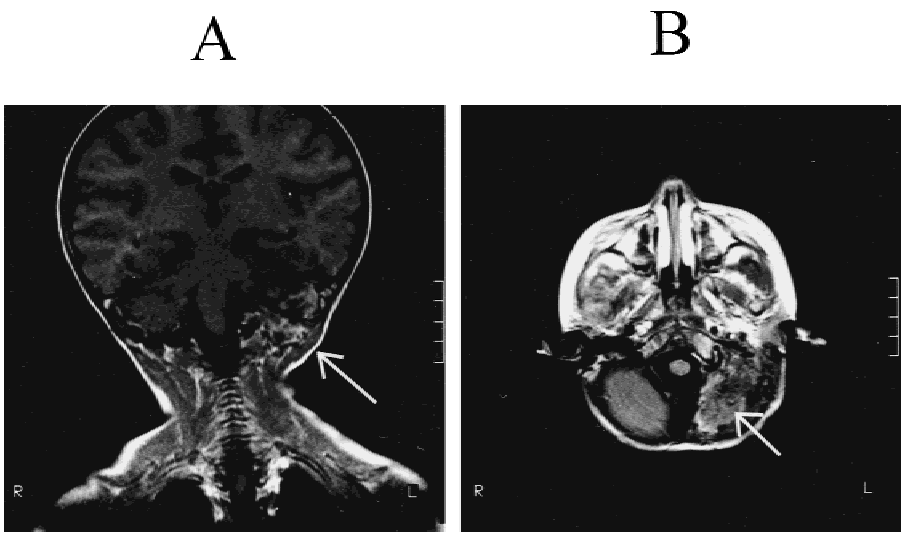

Fig. 1. Gadolinium contrast frontal (A) and axial (B) MRIs showed osteolytic lesions of left temporal bone and left skull base together with mild enhancement of left cerebellum mass. Enhancements of the adjacent dura and sinuses suggest slow venous flow around the cerebellum mass.

Christian disease and Hashimoto–Pritzker syndrome [10–17]. All these diseases, however, share a common feature of polymorphic infiltrates consisting of mononuclear and multinuclear Langerhans' cells in the afflicted area [10–17]. Elegant study by Arenzana–Seisdedos et al. [18] showed that the purified CD1a+ cells from patients with LCH can spontaneously produce interleukin 1 (IL-1) and prostaglandin E2 (PGE2). Lader and Flanagan [19] further show that the exogenous PGE2, IL-1, and TNFα can increase human osteoclast formation and bone resorption in vitro. Although the activated LCs and osteoclast may provide partial clue to the bone lesion of LCH, serum calcium level in these patients did not change markedly. Cause of osteolytic lesion in LCH remains to be determined.

In this report, we describe a 2-year-old boy with LCH in whom the disease manifestation was noted by the radiological evaluation with marked osteolysis of the right iliac bone, the left eighth rib, and the right mandible, as well as an enhancing mass in the left cerebellum. We further used immunocytochemical method to study the expressions of CD1a, S100, lysozyme, CD14, CD25, CD68, CD80, CD86, and Fas ligand (FasL) in the peripheral blood smear as well as bone marrow aspirate. Expressions of CD1a, S100, CD25, CD68, CD80, CD86, and FasL were identified on the Langerhans' cells.

CASE REPORT

A 2-year-old boy was referred to our hospital with a history of recurrent scalp sebarrhetic dermititis, bilateral otorrhea, as well as right mandibular and right hip swellings. Although no pain was complaint, a decreased willing to walk was noted. Otoneurological examination, on the other hand, was within normal limits, and audiological testing on both sides was intact. Scintigrams with 99mTc indicated the increased radioactivity in right mandible, right iliac, and right acetabulum. Abdominal echonograms revealed hepatomegaly, mild dilatation of left renal pelvis, and cavity lesion of right renal parenchyma. Radiological evaluation of head and neck by magnetic resonance imaging (MRI) and computed tomography (CT) showed osteolytic lesions of left temporal bone and left skull base together with a gadolinium contrast [20] mass in the left cerebellum (Fig. 1A and B). Erosion of the right mandible (Fig. 2A) with a soft tissue mass filling (Fig. 2B) was noted by X-ray radiography and CT. A similar condition was found in the inferior portion of the right iliac bone (Fig. 3A, B, and C). Surprisingly, although X-ray and CT indicated the complete absence of the left eighth rib, no soft tissue replacement was found (Fig. 4A and B). Laboratory evaluation revealed hemoglobin of 11.9 g/dl, and white blood cell count of 34,130/μl with 73.4% neutrophils, 17.9% lymphocytes, 8.8% monocytes, and 0.1% eosinophils. Platelet count was $506 \times 10^3/\mu l$. Serum electrophoresis and immunoelectrophoresis were normal, as were blood chemistries (calcium 10.0 mg/dl, glucose 139 mg/dl, ALT 23 U/l, and AST 12 U/l).

METHODS

Immunocytochemical study of CD1a, S100, lysozyme, CD14, CD25, CD68, CD80, CD86 (Dako, Carpenteria, CA), and Fas ligand (FasL, Transduction Laboratories, Lexington, KY) was performed on peripheral blood

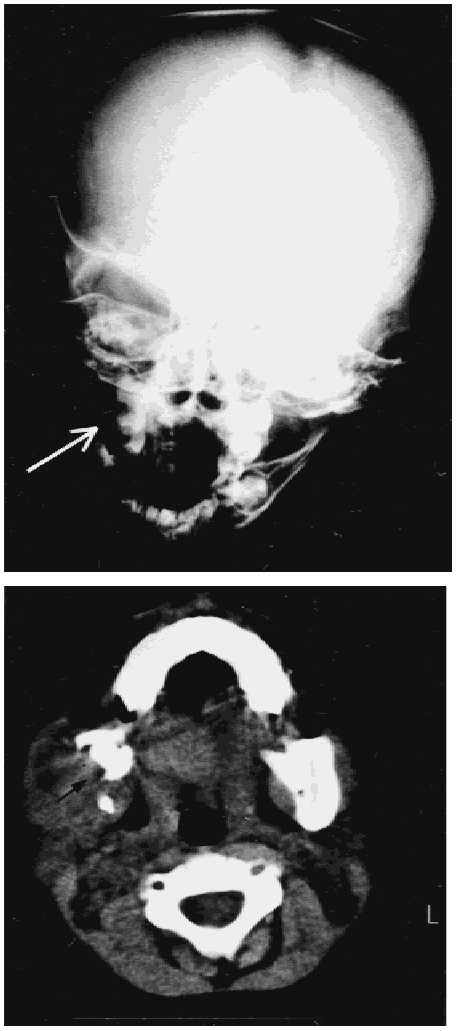

Fig. 2. (A) Frontal view of the skull by X-ray radiogram revealed osteolytic lesion of the right mandible (arrow). (B) CT image showed destruction of the right mandible and filling of the soft tissue mass (arrow).

smear and bone marrow aspirate by using the labeled Streptaridin-biotin (LSAB) method (Dako). The chromogenic reaction was visualized by peroxidase-conjugated streptavidin and aminoethyl carbazole (Sigma, St. Louis, MO). Slides were counter-stained with methyl green. Positive staining was recognized under the microscope as crimson granules. For the double staining, fluorescein-labeled antibodies (Benton-Dickinson, San Jose, CA) were added after the specimen was counter-stained. To detect viral gene product, immunocytochemistry with antibodies to LMP1, EBNA-2, and ZEBRA of Epstein-Barr virus (EBV) or to VP2 capsid protein of parvovirus B19 (PVB19) (Dako) or to late antigen of cytomegalovirus (CMV) (Novocastra, Newcastle-upon-Tyne, UK) was performed. In situ hybridization procedure [21] and polymerase chain reaction technique [22] were described previously. Probes synthesized by BRL (Bethesda, MD): 5′-fluorescein-GAGCTTATATAAGCCGAAAAAC-GTCTGAGATTCCTCA-3′, 5′-fluorescein-ATACTTCA-CTCCAGAAAGCAGGACAATTC-CATAGGTG-3′, and 5′-fluorescein-GAGCTGAAA-CAT-CCCCAGGCCCAATCCTACCAAGGCAACC-3′ were used to detect FasL expression. Chromogen was NBT/BCIP (Boehringer Mannheim, Germany). Cells from five cases of high-grade lymphoma without bone marrow involvement were used as controls.

RESULTS

Immunophenotyping on peripheral blood leukocytes was performed by using flow cytometry (FACSCalibur, Benton-Dickinson), showing the following results: Pan B (CD20) 56%; pan T (CD3) 45%, (CD4) 32%, (CD8) 13%, (CD14) 6.2%, (CD68) 7.6%, and (CD1a) 0.2%. In the peripheral blood smear, the frequency of CD68+ cells was 9.2%, and that of CD1a+ cells (Fig. 5A) was less than 1.0%. Interestingly, in addition to CD68+ cells, co-expression of FasL+ (Fig. 5B) was detected on most of the CD1a+ cells. Expression of FasL was further confirmed with ISH in these cells, and the same results were also obtained from the bone marrow aspirate as well as bone marrow biopsy (data not shown). Results of immunocytochemistry are listed in Table I with ten cases of histiocytic necrotizing lymphadenitis (HNL) and three cases of infection-associated hemaphagocytic syndrome (IAHS) [1,11–17,23] studied at the same time.

DISCUSSION

The results presented above show that the Langerhans' cells from this patient expressed CD1a and FasL. This conclusion is based on the presence of both markers on the same Langerhans' cell as detected by combining immunocytochemistry and immunoflurescence microscopy. Previous reports have described patterns of LCH with expressions of CD1a, S100, CD24, CD68, CD80, lysozyme, and Birbeck granules that suggest activated LCs in the disease [24]. Recently, by using X-chromosome inactivation assay of human androgen receptor gene, Yu et al. [25] and Willman et al. [26] indicated that LCH is a clonal histiocytic disorder. Nonetheless, they argued that although the principal feature distinguishing a neoplasm from a reactive process is monoclonality of the cell, the clonality alone was not able to fully interpret the difference in severity and progression of the disease.

From immunohistochemical data, Ruco et al. [12] suggested that the heterogeneous Langerhans' cell population in Letterer–Siwe disease could indicate the immature LCs of unknown cause. Several studies [18,27–31] supported the above hypothesis by showing that LCH correlated with functional defect of LCs in these patients.

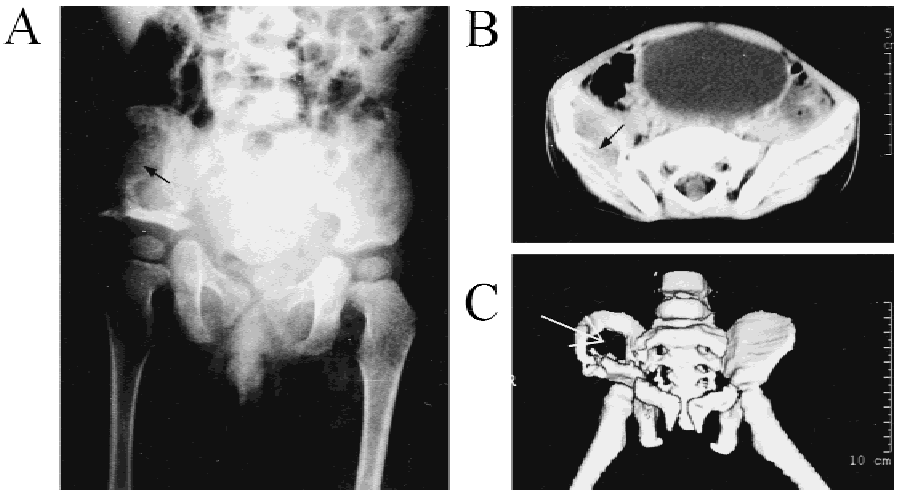

Fig. 3. Damage of the right iliac bone. (A) LCH involving the right iliac bone was shown by X-ray; (B) CT image showed the soft tissue mass (arrow); (C) A three dimension of CT image showed the damage of the right iliac bone (arrow).

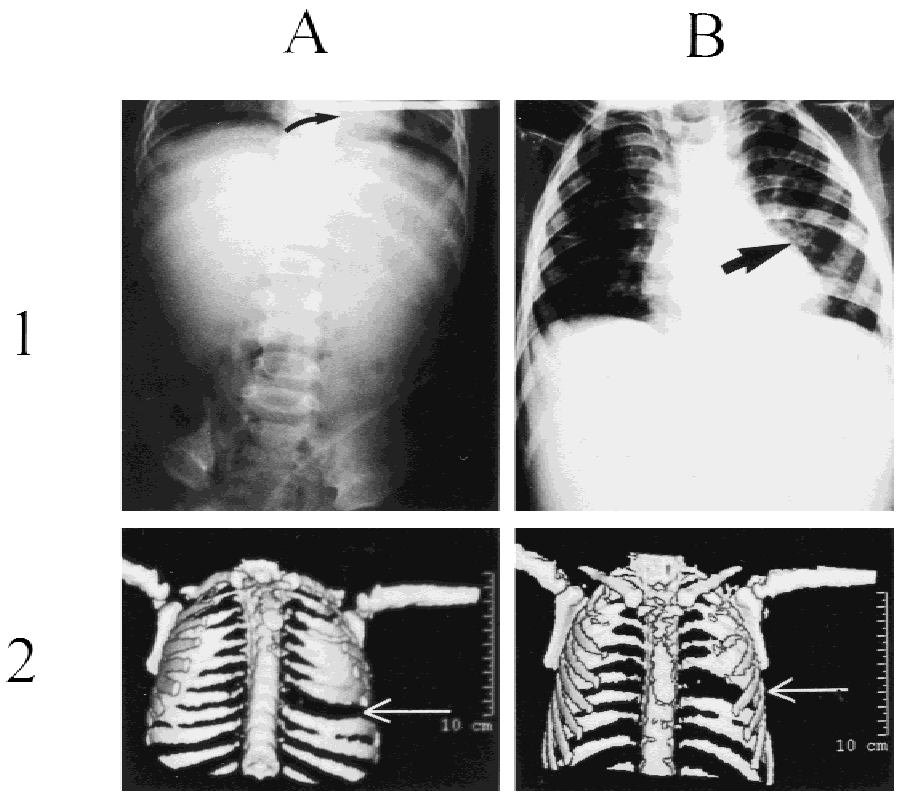

Fig. 4. Osteolytic lesion of the left eighth rib. (Row 1, A) The left eighth rib (arrow) was intact when the boy was 5 month-old. (Row 1, B) Complete destruction of the left eighth rib (arrow) was shown when the boy was 30 month-old. (Row 2, A) A three dimension CT image showed the absence of left eighth rib. (Row 2, B) Ten degree sagittal tilt of a three dimension CT image as shown in Row 2, A.

They further showed that functional defect could involve the decreased antigen-presenting activity [27,28], lack of E-cadherin expression [29], cytokine dysregulation [18,30] and the decreased number of late differentiating suppressor lymphocytes, namely CD8+ cells [31].

As mentioned previously, purified CD1a+ cells from patients with LCH could spontaneously produce IL-1 and PGE2. Because the exogenous IL-1 and PGE2 could enhance human osteoclast function and bone resorption, there is a good reason to believe that activated LCs could induce the osteolytic lesion in LCH. Nevertheless, no hypercalcemia was detected in patients with LCH. In

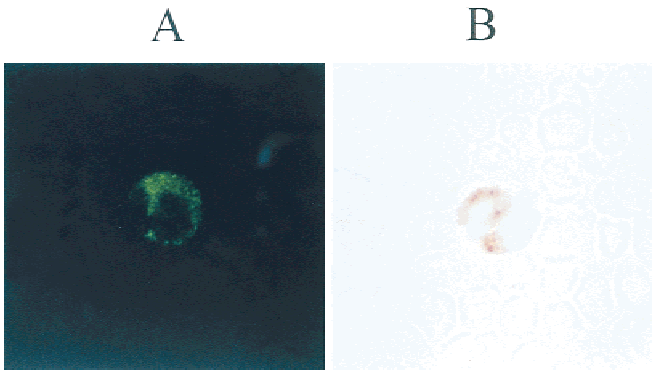

Fig. 5. Expressions of CD1a and Fas ligand in the Langerhans' cell of peripheral blood smear from patient with LCH. By immunofluorescence microscopy, CD1a was detected on Langerhans' cell (A), and by immunocytochemistry, co-expression of FasL (B) was detected.

TABLE I. Immunostain Results of Patients with LCH, HNL and IAHS

Marker	LCH (n = 1)	HNL (n = 10)	IAHS (n = 3)
CD1a	+	+[a]	+
S100	+	−	+
Lysozyme	−	+	+
CD14	+	+	+
CD25	+	+	+
CD68	+	+	+
CD80	+	+	−
CD86	+	+	−
Fas ligand	+	+	+[b]–
CMV	−	−	+[b]/–
EBV	−	+	−
PVB19	−	−	+

[a]$CD1a^+$ signals were identified on sinusoid cells.
[b]Signals of FasL and CMV were only detected in a case with AIDS.

addition, our results showing the absence of left eighth rib without LC mass filling and bone pain in the patient would argue that increased osteoclast activity is solely responsible for the bone damage. Recent studies by Kawakami et al. [32] and Jilka et al. [33] clearly demonstrate that human osteoblast expresses Fas (Apo-1/CD95), and interaction between Fas and FasL could mediate osteoblast apoptosis. Constitutive expression of FasL was also detected on multiple myeloma cells that were frequently associated with osteolytic lesion and hypercalcemia [34,35]. It is worth noting that from the "local coupling" theory of bone formation and resorption the intact osseous configuration is kept by the concerted activities of osteoblast and osteoclast [32,33]. Although a malfunction of either cell type may jeopardize rigidity and structure of the bone, the complete damage requires the increased activity of osteoclast and the decreased function of osteoblast. The study of this case considered together the others [2–19] clearly indicates that LCs could express IL-1, PGE2 and FasL at the same time. The exact role of each feature in producing the syndrome, however, remains to be determined.

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